## Remarks

The Office Action mailed February 14, 2005 has been received and carefully reviewed. Claims 44, 50, 56, 64-73 and 78-80 having been amended, the pending claims are claims 44-48, 50-54, 56-60 and 64-80. Reconsideration and withdrawal of the rejections are respectfully requested.

## Examiner Interview Summary

The Examiner is thanked for the telephonic interview conducted on July 8, 2005. Participating were Examiner L. E. Crane, Examiner Michael Woodward (Quality Assurance Specialist) and Applicant's Representative Victoria Sandberg. All claims were discussed although complete agreement was not reached.

It is noted that Applicant's Representative has not yet received an Interview Summary from the Examiner for the interview conducted on July 8, 2005. An Interview Summary was, however, mailed by the Office on July 6, 2005, describing Applicant's Representative's telephonic request for said interview. Applicant concurs with the Examiner's description of the request for interview set forth in the Examiner's Interview Summary mailed July 6, 2005.

During the interview held on July 8, 2005, Applicant's Representative reiterated Applicant's position that the objection to the specification for improper incorporation by reference of essential material is not appropriate without an identification by the Office as to what material is considered to be essential. Examiner Woodward indicated that it is the responsibility of the Office to identify the essential material that is allegedly improperly incorporated by reference.

Also discussed were the rejections under 35 U.S.C. §112, first paragraph, as they relate to the method of the invention utilizing probes that hybridize to the template nucleic acid at positions that produce a gap or an overlap between the two probes, and also as they relate to a probe containing less than 7 nucleotides. Examiner Crane indicated that working examples of these embodiments showing successful autoligation would be helpful, and suggested the

Applicant provide a Declaration under 37 C.F.R. §1.132 setting forth experimental results. Applicant has considered this option but upon further review of the Office Action and the disclosure believes the Examiner's rejections can be addressed and overcome based upon the arguments of record and additional arguments set forth herein.

Also discussed was the rejection under 35 U.S.C. §112, second paragraph, as it relates to the recitation of the term "comprising" in the claims. Applicant's Representative pointed out the differences between a small molecule chemical structure, and a polymeric structure such as an "oligonucleotide" (as recited in the claims), and argued that the use of the open-ended term "comprising" satisfies the requirements of 35 U.S.C. §112, second paragraph, in the latter case. The Office agreed to reconsider the Applicant's arguments in this light.

## Objection to the Specification

The specification remains objected to for improper incorporation of essential material by reference to a foreign application or a foreign patent or to a publication, and the Examiner continues to require that the disclosure be amended to include the material incorporated by reference. This objection is respectfully traversed.

"'Essential material' is defined as that which is necessary to (1) describe the claimed invention, (2) provide an enabling disclosure of the claimed invention or (3) describe the best mode (35 U.S.C. 112)." MPEP 608.01(p)(I)(A).

Incorporation by reference of both essential material and nonessential subject matter is expressly permitted by MPEP 608.01(p)(I)(A), under conditions described therein. With respect to essential material, an attempt to incorporate it into a specification by reference to a document other than (1) a U.S. patent, (2) a U.S. patent application publication, or (3) a pending U.S. application would be improper according to MPEP 608.01(p)(I)(A).

The Examiner has failed to describe with any particularity what constitutes the "essential material" that the Applicant is attempting to improperly "incorporate by reference." In addition to failing to identify any material that is *essential,* the Examiner has further failed to show that

any allegedly essential material is *described in a referenced document* other than a U.S. patent, U.S. patent application publication, or pending U.S. application such that it is improperly incorporated into the present specification by reference.

Applicant submits that the specification does *not* improperly incorporate by reference any material necessary to (1) describe the claimed invention, (2) provide an enabling disclosure of the claimed invention or (3) describe the best mode (35 U.S.C. 112). No such improperly incorporated "essential material" has been identified by the Examiner. Furthermore, the general "incorporation by reference" language recited at page 61, lines 13-19 of the specification is proper since it is permissible to incorporate nonessential subject matter into the specification pursuant to MPEP 608.01(p)(I)(A).

Reconsideration and withdrawal of the objection to the specification is, accordingly, respectfully requested.

## Rejection under 35 U.S.C. §112, First Paragraph

The Examiner newly rejected claims 44-48, 50-54, 56-60, and 64-80 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a Written Description rejection. This rejection is respectfully traversed.

The Examiner first alleges that the claims have not met the written description because the specification has failed to note the "criticality of the temperature in the execution of the autoligation process." The Examiner emphasizes temperature as a "critical variable" when the associated hybridization process is being relied upon to distinguish between matched and mismatched hybridization outcomes, which the Examiner characterizes as an "essential part of the instant claimed detection process."

Applicant disagrees. MPEP 2163 (directed to the Written Description requirement) states that the claimed invention as a whole may not be adequately described if the *claims require an essential or critical feature which is not adequately described in the specification and which is not conventional in the art or known to one of ordinary skill in the art* (emphasis added).

To begin with, temperatures for autoligation are *described* at numerous places in the specification, all of which involve working examples. The following is an exemplary list of reaction temperatures, where page and line numbers refer to an exemplary location for this temperature in the instant specification:

| | |
|---|---|
| 20°C | page 39, line 2; |
| 23°C | page 26, line 27; |
| 25°C | page 40, line 4; |
| 37°C | page 38, line 29; |
| 50°C | page 47, line 5; |
| 70°C | page 39; line 25. |

Further, Applicant maintain that the temperature of the autoligation process is decidedly not a *critical or essential feature* of the invention that is required by the claims. To the contrary and as evidenced by the wide *range of temperatures* indicated above, the autoligation reaction, which is carried out over several hours, successfully produces an autoligated product over an entire range of reaction temperatures. Hybridization is indeed temperature-dependent, in that it is well-known to the art that a reaction temperature must be below the Tm of the duplex in order for the strands to hybridize, but a particular reaction temperature for the autoligation process is not necessary, hence the temperature is not an essential or critical feature. Tm's can be readily calculated for any nucleotide sequence using widely available algorithms.

With respect to the particular case of *distinguishing between matched and mismatched* hybridization outcomes, Applicant begins by noting that such a step is only recited in claims directed to methods that make use of both a mutant probe and a wild-type probe; namely, claims 50-54; claims 67-69; and claims 73-80. This aspect of the rejection is thus applicable only to those claims.

Distinguishing between matched and mismatched hybridization outcomes is described, for example, in Example III of the present specification, which reports experiments designed to test the effect of probe + target concentration and temperature on the extent and rate of ligation of complementary (matched) and mismatched template-directed autoligation according to the invention (specification at page 38, lines 10 through 25).

The figures show discrimination between matched and mismatched probes in a number of different circumstances, as noted below, for example:

| | |
|---|---|
| Fig. 9a | 25°C; linear probes, 20uM probe |
| Fig. 9b | 25°C; linear probes, 1.3uM probe |
| Fig. 9c | 37°C; linear probes, 1.3uM probe |
| Fig. 10a | 25°C; cyclization probe, 1.3uM probe |
| Fig. 10b | 70°C; cyclization probe, 1.3uM probe |
| Fig. 11b | 70°C; cyclization probe |
| Fig. 12 b | 37°C; linear probes |
| Fig. 14a | 25°C; linear probes |

Only in one of these cases (Fig. 10a; specification at page 40, lines 4-8) do the amount of the matched and mismatched ligation products approach each other, and they are still quite distinguishable. The choice of temperature is not critical, and the specification gives clear guidance in many detailed working examples as to conditions, including temperature, for carrying out the autoligation reaction.

The Examiner cites Northwestern University '699 (PTO-1449 ref. AM) as evidence that temperature was found to be a "critical variable" for effective autoligation when the associated hybridization process is being relied upon to distinguish between matched and mismatched hybridization outcomes. Applicant again disagrees. The autoligation experiments in Northwestern University '699 that involved matched and mismatched were conducted only at two different temperatures, 0°C and 30°C, and only for a very short period of time–only five minutes (Northwestern University '699 at page 24, lines 5-11). All that Northwestern University '699 teaches in this regard is that in a 5 minute autoligation reaction at 0°C, conversion of the ligated product amounted to 85% for the matched template, and 78% and 74% for mismatched templates. This still represents a level of discrimination, and certainly does not prove that temperature is a "critical variable" for the functionality of the autoligation method of the Northwestern University '699, much less the instantly claimed method. Moreover, one of ordinary skill in the art would know that carrying out a hybridization reaction at 0°C would substantially decrease the stringency of hybridization and compromise the sensitivity of the method. Indeed, this is exactly what is taught in the prior art, in Northwestern University '699.

Second, the Examiner suggests that the instant claimed process wherein three probes are present is closely analogous to a combination of the "matched" and "mismatched" examples disclosed by Northwestern University '699, but fails to show how this variation produces a different result. In addition, the Examiner alleges that the instant description fails to disclose the advantages of conducting the three probe process or how the results may be interpreted based on how these particular probes and their labels are selected. Applicant disagrees.

Applicant again notes at the outset that this aspect of the rejection only applies to the claims directed to methods that make use of both a mutant probe and a wild-type probe; namely, claims 50-54; claims 67-69; and claims 73-80.

Further, it is unclear what aspect of the claims the Examiner is referring to, and how the Examiner's comments are to be interpreted in the context of a Written Description rejection, in

contrast to a rejection based on the prior art. Many differences between the teachings of Northwestern University '699 and the method recited in the pending claims were pointed out by Applicant in the most recent Amendment mailed September 21, 2004 (in the context of a rejection under 35 U.S.C. §102), and this argument was sufficient to overcome the rejection under 35 U.S.C. §102. It is not now clear how the same art is being used in a rejection under 35 U.S.C. §112, first paragraph.

Applicant submits that it is not necessary for the method of the claimed invention to produce a "different result" from Northwestern University '699 because it is a different method, as discussed in detail in the most recent Amendment mailed September 21, 2004. Further, 35 U.S.C. §112, first paragraph, does not require that the Applicant describe the "advantages" of the process; it is sufficient if the invention has been described with sufficient particularity such that one skilled in the art would recognize that the Applicant had possession of the claimed invention.

The Examiner further asserts that the description fails to disclose "how the results [of the three probe process] may be interpreted based on how the particular probes and their labels are selected." Applicant disagrees. Example IV, for example, describes studies carried out with three probes simultaneously, each probe carrying a different fluorescence donor or acceptor (specification at page 44, lines 18-20). This example describes a three probe autoligation method and the experimental results thereof at pages 49-51 of the specification. Yellow, red or green signals reflecting the different autoligation products were observed as a result of fluorescence energy transfer. The products of the autoligation reactions could also be separated using gel electrophoresis (specification at page 51, lines 3-15).

Finally, the Examiner states that "hairpin formation" is the necessary result of either a 1 or 2 nucleotide overlap or a 1 or 2 nucleotide gap situation, and asserts that this has not been addressed by the instant disclosure of by the art of record. Lehninger (PTO-892, ref. **U**) is cited.

The Examiner elaborated on this rejection in the telephone interview, questioning whether autoligation will occur under such circumstances, since the specification contains no working examples that involve a gap or an overlap in the binding of the two probes. For the reasons described below, Applicant submits that the specification provides the requisite written description, and one of skill in the art would recognize that the Applicant had possession of the invention as claimed.

Applicant first wishes to clarify the terminology used by the Examiner. "Hairpin" DNA contains inverted repeats; the two adjacent strands are held together by base-pairing. See the Oxford Dictionary of Biochemistry and Molecular Biology, Oxford University Press, 1997. Embodiments of the present method that are practiced using a 1 or 2 nucleotide overlap or a 1 or 2 nucleotide gap situation do not product a hairpin as an autoligation product, nor does the template form a hairpin.

However, a 1 or 2 nucleotide overlap or a 1 or 2 nucleotide gap situation does suggest the presence, in either the autoligation product or the template, of a 1 or 2 nucleotide structure that is known to the art as a "bulge loop" or simply a "bulge." A bulge is a structure in a polynucleotide duplex in which one strand contains a sequence of one or more nonterminal nucleotides that are not able to base pair with the other strand. See the Oxford Dictionary of Biochemistry and Molecular Biology, Oxford University Press, 1997.

The inventors note in the specification that the autoligation process is very forgiving concerning the steric conditions at the autoligation site. That is, strict stereochemical conditions are not needed for autoligation to occur. At page 42, lines 4-14, in discussing the differences in selectivity for the various matched and mismatched probes tested, the specification states:

> It is of interest of consider the physicochemical origins of the selectivity for this autoligation reaction. The data show that selectivity is lower at the ligation junctions but higher near the center of a short probe. We surmise, therefore, that *the chief factor in successful ligation is the binding affinity of the probe rather than the precise geometry at the ligation junction.* This is consistent with our finding that the reaction proceeds with second-order kinetics. *In contrast, enzymatic ligations are commonly most selective at the junction*, a fact which is

> attributed to the precise geometric control that the enzyme takes in orienting the reactive groups for in-line attack at phosphorus (N. Higgins et al., Meth. Enzymol., 68, 50-71 (1979)). *For the present reaction it seems that the transition state $S_N2$ geometry can be reached even with mismatched geometries; this is likely due to the relatively high flexibility of the DNA at the nicked junction.* Presumably, ligase enzymes curtail this mobility to a high degree (emphasis added).

This means that a mismatched probe will autoligate more readily if the mismatch is at the ligation junction than if the mismatch is internal to the probe. Experiments set forth in Example III indicate that even when a mismatch (MM) occurs on the 5' end of the downstream probe (see SEQ ID NO:28 in Fig. 8, denoted 5'MM target), a significant amount of autoligation product is produced (see Figs. 9a, 9b and 9c). The fact that autoligation occurs via a chemical $S_N2$ reaction instead of enzymatically means that stereochemistry is not as important in autoligation according to the claimed invention as it is to an enzymatic reaction. Further, the DNA/RNA template will be flexible at the "nick" site that is created by the hybridization of the upstream and downstream probes to the template, thereby facilitating the autoligation reaction by *twisting or bending* to put the two reactive groups into closer proximity to each other (see passage cited immediately above; see also Roll et al., Biochemistry 1998, 37, 4059-4070, and Mills et al., Biochemistry 1994, 33, 1797-1803, abstracts describing flexibility at the site of a nick or a gap). Further, as discussed below, post-filing art shows that autoligation does indeed occur in template-promoted self-ligation reactions using oligonucleotide probes that bind to a template and produce a gap or overlap, thereby generating a bulge in the ligated product or the template (Abe et al., J Am. Chem. Soc. 2004, 126, 13980-13986, e.g., first full paragraph at page 13982; Ihara et al., J. Am. Chem. Soc. 126(29), 8880-8881, 2004). Thus, even though no working examples of autoligation reactions that involve gaps or overlaps are included in the specification, the specification clearly teaches *why* this embodiment of the invention is expected to be functional; this expectation *is* reasonable in view of the art; and it would be quite evident to one of skill in the art that the inventor had possession of the invention as claimed.

For at least the reasons set forth above, it is respectfully submitted that the subject matter contained in the claims is described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention, and that the Written Description requirement under 35 U.S.C. §112, first paragraph, is met.

The Examiner also newly rejected claims 44-48, 50-54, 56-60, and 64-80 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. This rejection is respectfully traversed.

The Examiner states that the issue is whether practicing the full scope of the invention is possible without undue experimentation. Particular subject matter that the Examiner asserts is not enabled is identified in the Examiner's discussion of the particular *In re Wands* (858 F.2d 731, 737; 8 USPQ2d 1400, 1404, Fed. Cir. 1988) factors. These factors include (1) quantity of experimentation necessary; (2) the amount of direction or guidance presented; (3) the presence or absence of working examples; (4) the nature of the invention; (5) the state of the prior art; (6) the relative skill of those in the art; (7) the predictability or unpredictability of the art; and (8) the breadth of the claims,

A. Breadth of the claims

The Examiner asserts that the breadth of the claims is excessive because the instant disclosure fails to provide an enabling description of how autoligation can be carried out when the resultant hybridization must produce a product with a "hairpin" (i.e., a bulge) due to a gap or overlap of the probes. Applicant disagrees, and asserts that the specification is enabling for this aspect of the invention. Autoligation was shown to occur when the base pair mismatch occurred

on the end of a probe at the site of autoligation, providing evidence that the stereochemical requirements for autoligation are very relaxed (e.g., specification at page 42, lines 4-14).

Moreover, the specification is very clear in stating that the probes which hybridize to yield a 1 or 2 base gap or overlap are expected to autoligate, a conclusion that is certainly reasonable to one of skill in the art given the experimental observations just described. As additional support for the reasonableness of this expectation, Applicant submits post-filing art (Abe et al., J Am. Chem. Soc. 2004, 126, 13980-13986) showing that autoligation occurs in template-promoted self-ligation reactions using oligonucleotide probes that bind the template to produce a gap or overlap, whether in an autoligation reaction involves a 5' leaving group (e.g., dabsyl) reacting with a 3' terminal carbon, e.g., Abe et al., first full paragraph at 13982) or in an autoligation reaction that utilizes an intentionally destabilizing linker design in the probe that contains the 5' leaving group, (e.g., Abe et al., second full paragraph at page 13892). See also Ihara et al. (J. Am. Chem. Soc. 126(29), 8880-8881, 2004) which, although it describes a different autoligation chemistry, shows that template-directed autoligation occurs when the oligonucleotides bind to yield a 1-3 nucleotide gap (e.g., Ihara et al., Table 1 at page 8881).

The Examiner further asserts that the instant disclosure fails to explain how the desired result may be obtained without careful consideration of the temperature at which the process is conducted. Applicant again disagrees, and directs the Examiner's attention to the discussion of temperature set forth above in response to the Written Description rejection. To recap, temperature is not critical; the desired result can be obtained over a wide range of temperatures, and a wide range of temperatures is specifically described in the working examples.

The Examiner further asserts that there are no disclosures in the claims directed to the particular process conditions required to effect "concomitant autoligation with hairpin formation" which the Examiner describes as a necessity associated with the presence of either a gap between, or overlap of, the termini of the two probes which have successfully hybridized with the target oligonucleotide. Applicant responds that the process conditions taught in the specification provide sufficient guidance to one of skill in the art, no special process conditions

are needed, and that experimental variation(s), if any, to effect autoligation in such circumstances would be well within the skill of one skilled in the relevant art. The experimental observations that support this conclusion as reasonable to one of skill in the relevant art are described above in connection with the response to the Written Description rejection.

The Examiner further asserts that there are no disclosures of the conditions required to achieve the claimed utility of the hybridization when one probe is, as specified in claim 50, less than 7 nucleotides in length, a variation on the prior art which the Examiner alleges is unknown when the ligation process is not enzymatic. Also, in section "E." of the Office Action regarding the level of predictability of the art, the Examiner expresses concerns about the hybridization being under high stringency conditions, which will be addressed here as well for completeness.

Applicant responds that the autoligation conditions taught in the specification are effective for probes of any length as described in the claimed invention. In fact, a probe containing 6 nucleotides *is taught in a working example.* This is the cyclization probe shown in Fig. 11a (SEQ ID NO:31) (specification at page 40, line12, stating "6 bp on one side of the junction and 14 bp on the other side."). The 5' probe sequence thus contains only 6 nucleotides, and autoligation proceeds without any difficulty (Fig. 11b). Template directed autoligation of two hexamers is also described in Ihara et al. (J. Am. Chem. Soc. 126(29), 8880-8881, 2004), although a different chemistry (photochemical ligation) is utilized.

Moreover, it is well within the ordinary skill of one skilled in the art to calculate the Tm for any desired probe based upon its sequence (no experimentation is needed), and conduct the autoligation at a temperature that is below the Tm for that probe. Optimization of hybridization conditions are well within the ordinary skill of one skilled in the art, and many examples of optimizations of conditions are taught in the working examples.

The experimental results described in the specification show that shorter probes are very effective: "when the mismatch fell within the shorter binding domain [6bp], the selectivity was increased" (specification at page 40, lines 16-17). In discussing the advantages of autoligation over enzymatic approaches, the specification states that autoligation "can be used with short

probes such as heptamers, which cannot be ligated by the commonly used *Tth* ligase" (specification at page 43, lines 8-10). In subsequent experiments (e.g., Example IV), 7mer and 13 mer probes were used "which can afford very high sequence specificity due to the high mismatch selectivity of the shorter of these probes (specification at page 44, lines 10-11). "Two 7mer probes (mutant and wild-type) were designed such that the position of the point mutation fella the center position, to maximize selectivity" (specification at page 44, lines 12-14). This was also recognized in the statement at page 18, lines 22-25, wherein the specification states:

> An oligonucleotide probe of the invention is preferably between about 3 and about 12 nucleotides in length, more preferably between about 4 and about 8 nucleotides in length. Significantly, this process allows very short SNP oligonucleotide probes to be used, e.g. those of 3, 4, 5, and 6 nucleotides in length.

It is clear that 7mer probes function very well in the claimed method and there is no evidence in the specification or provided by the Examiner to support the contention that shorter probes would not also function in the claimed method; indeed the specification supports the opposite conclusion. One of skill in the art would have no reasonable basis to believe that probes shorter than 7 bases would not work in the claimed method, and in fact a 6mer was shown to be effective in a working example.

B. Nature of the invention

The Examiner discusses "hairpin" (bulge) formation, and short (less than 7 nucleotides in length) probes, both of which are addressed above.

The Examiner further remarks that there is no disclosure in the claims as to how "detecting the presence of the autoligated product" is to be accomplished. Applicant counters that methods of detection of oligonucleotides are well known to the art and as such do not need to be recited in the claims. Numerous examples of detection methods are mentioned in the specification (e.g., using a detectable label such as detectable label can be a radiolabel, a

fluorescent label, a chemical label, an enzymatic label, an affinity label, or the like; or using fluorescence energy donors and acceptors; an excimer label; or gel electrophoresis). See, e.g., the specification at page 6, lines 17-23; page 15, lines 5-8; and page 22, lines 3-11. Working examples utilize, for example, radiolabels (e.g., specification at page 47, line 28-29), fluorescent labels (e.g., specification at page 56, lines 1-5) and fluorescence resonance energy transfer (FRET) labels (e.g., specification at page 50, lines 4-15). In the three probe process, the specification makes it clear that the different autoligation processes can be differentiated, for example by differentially labeling the mutant and wild-type probes (specification at page 23, line 2 bridging to page 24, line 3). Furthermore, since mutant and wild-type polymorphism oligonucleotide probes may be the same length or differ in length (specification at page 18, lines 25-27), resulting in autoligated products of different lengths that are thus distinguishable using physical methods as well ("ligation events also can be detected using PCR, rolling circle amplification, gel electrophoresis or the like without detectably labeling the ligation products"; specification at page 19, lines 13-15).

C. State of the prior art

The Examiner notes that an overlap/gap based autoligation "testing protocol" is unknown to the art, and the there is no teaching in the prior art of autoligation having been successfully applied with probes of less than 7 nucleotides in length. Applicant responds that these aspects of the claimed invention are novel and thus not in the prior art; and further present disclosure that the disclosure adequately enables both aspects of the invention for reasons discussed above.

D. Relative level of skill of those in the art

The Examiner states the level of ordinary skill is high with regard to "directly adjacent autoligation" (citing Northwestern University '699) but "very low" with regard to overlap, gap and/or short (<7 nucleotides) probe based autoligation. Applicant takes issue with this statement. The skill level in the art is the same whether the artisan is conducting a directly

adjacent autoligation or an autoligation involving a gap or overlap between the probes. This *Wands* factor refers to an *overall* skill level in the relevant art, and the relevant art is the art of chemistry and biochemistry. It is well-recognized that the level of skill in the art of chemistry and biochemistry is high. The fact that the present invention is directed to novel subject matter (which it needs to be in order to be patentable) does not render the skill level of an artisan in the relevant art "low."

E. The level of predictability in the art

As with the skill level factor, the Examiner erroneously bases the evaluation of predictability on whether the particular subject matter is known to the art (making it predictable) or whether it is unknown to the art (making it unpredictable). The relevant art is the art of chemistry and biochemistry. Applicant asserts that the level of predictability in the art of chemistry and biochemistry has become fairly high over the last several decades. For example, based upon the experimental results reported in the instant specification, Applicant asserts that one of skill in the art would reasonably expect the claimed processes to function with probes having less than 7 nucleotides and probes the resulted in a gap or overlap prior to the autoligation reaction (for reasons described above). Similarly, hybridization conditions are highly predictable based on the nucleotide sequence of the probe(s), and it is well within the ordinary skill of one in the relevant art to identify such conditions.

F. The amount of direction or guidance presented

The Examiner states that the direction provided by the inventor is very high for directly adjacent probes but "non-existent" when the adjacent probes are separated by a gap or overlap each other, or when the probes are less than 7 nucleotides in length. Applicants disagree. One of skill in the art, by practicing the invention as claimed and with reference to the teaching of the specification, can reasonably expect to achieve autoligation in all three of these cases. As described in more detail above, autoligation is expected to occur in the case of a gap or overlap

in accordance with the method as claimed because the chemistry does not require a strict stereochemistry, and the template retains flexibility at the "nick" site. Autoligation is expected to occur in the case of a probe less than 7 nucleotides in accordance with the method as claimed because the identification of hybridization conditions are well within the skill of one of ordinary skill in the art. Additionally, numerous working examples of 7mer probes, and one working example of a 6mer probe, are taught.

G. The existence of working examples

The Examiner states that the working examples are limited to directly adjacent autoligation examples, and states further that no examples of autoligation with a probe of less than 7 nucleotide units under low stringency hybridization conditions are present. Applicant responds by noting that the specification contains an extensive array of working examples directed to different types of probes (e.g., linear and circular); different lengths of probes; different sequences of probes; differentially labeled probes; different autoligation conditions; and the like. There is also a working example that specifically includes a 6 nucleotide probe (specification at page 40, lines 9-21).

H. The quantity of experimentation needed

The Examiner states that the quantity of experimentation needed to make or use the invention based on the content of the disclosure is excessive and therefore undue when the abutting target-sequence-hybridized probes have either a gap separating them, are overlapping, and /or are less than 7 nucleotides in length. Applicant disagrees, and asserts that the amount of experimentation needed is minimal, if needed at all. For reasons at least the reasons outlined above in connection with the other *Wands* factors, the autoligation reaction would expected by one of skill in the art to proceed with a gap or overlap between the probes without any substantial modification of the reactions conditions described in the specification. Likewise, the autoligation reaction would expected by one of skill in the art to proceed with an oligonucleotide

probe having less than 7 nucleotides without the need for any undue experimentation. Hybridization conditions for the shorter would be easily discernable by one of skill in the art. In this regard, see Exhibit C (Sambrook and Russell) submitted with the Amendment mailed September 21, 2004, and listed on the 1449 form submitted on even date therewith and initialed as considered by the Examiner (reference ES) on December 19, 2004.

For at least the foregoing reasons, it is respectfully submitted that claims 44-48, 50-54, 56-60, and 64-80 meet the requirements of 35 U.S.C. §112, first paragraph. Reconsideration of the rejection under 35 U.S.C. §112, first paragraph, is respectfully requested..

**Rejection under 35 U.S.C. §112, Second Paragraph**

The Examiner newly rejected claims 44-48, 50-54, 56-60 and 64-80 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. The rejection is respectfully traversed.

Specifically, claims 44-48, 50-54, 56-60 and 64-80 are rejected under 35 U.S.C. §112, second paragraph, as being incomplete for omitting essential elements, namely, the criticality of the temperature of the process. MPEP 2172.01 is cited, as is Northwestern University '699 at pages 23-25. For reasons set forth above, Applicant disagrees that temperature is an essential element of the invention.

Moreover, MPEP 2172 is entitled "Subject Matter Which the Applicants Regard as Their Invention" and states that an rejection based on failure to satisfy this requirement is appropriate only where *Applicant* has stated, somewhere other than in the application as filed, that the invention is something different from what is defined by the claims. In other words, the invention set forth in the claims must be presumed, in the absence of evidence to the contrary, to be that which the Applicants regard as their invention. MPEP 2172. This is a subjective requirement, based upon what the Applicant regards as the invention. MPEP 2171. The

Examiner has not provided any evidence that the Applicant has stated that temperature of the process is critical. The Examiner's citation of Northwestern University '699 for the allegedly criticality of the temperature of the process does not provide any evidence that the *Applicant* has disclosed this element to be essential.

Claims 44-48, 50-54, 56-60 and 64-80 are rejected under 35 U.S.C. §112, second paragraph, as being incomplete for omitting essential steps, namely, the experimental variation(s) required to insure that autoligation occurs when there is either a gap of 1 or 2 nucleotide units or an overlap of 1 or 2 nucleotide units. Again, MPEP 2172.01 is cited. For reasons set forth above, Applicant do not consider any experimental variation(s), if any, needed to insure that autoligation occurs when there is a gap or an overlap as described to be essential steps. Such experimental variation(s), if any, are well within the skill of one skilled in the art. Furthermore, as noted in the preceding paragraph, MPEP 2172 relates to a subjective requirement, and the Examiner has not provided any evidence that the *Applicant* has stated such experimental variation(s), if any, are essential steps.

Claim 44 at line 13 and claim 65 are rejected under 35 U.S.C. §112, second paragraph, for allegedly being indefinite in that the phrase "not directly adjacent to" is not defined in the disclosure. Applicant respectfully disagrees.

At page 16, lines 7-12, the specification states that:

> The term "substantially adjacent" thus includes, for example, oligonucleotides that are bound to the template or target polynucleotide directly adjacent to each other; oligonucleotides bound to the template or target polynucleotide such that there is a gap of 1 or 2 bases between the "upstream" and "downstream" oligonucleotide; and oligonucleotides bound to the template or target polynucleotide such that there is a 1 or 2 nucleotide overlap between the two oligonucleotides.

Clearly, probes are "directly adjacent" when they bind to a template such that there is no gap or overlap. It necessarily follows that probes are *not* directly adjacent when they bind to a polynucleotide template such that a gap or overlap is produced.

Claim 50 is rejected under 35 U.S.C. §112, second paragraph, for allegedly being unclear and/or incompletely defined because the instant process had not provided a clear description of how a minimum of two different possible products, or the mixture of the two or more different products, are separately detected. Applicant respectfully disagrees.

Applicant submits that the Examiner's statement that a mixture of multiple different target polynucleotide sequences may generate multiple different products represents a misunderstanding of the invention. The number of possible products depends solely on the number of probes used. The production of any given autoligation product indicates that its associated polynucleotide template is present in the sample. Each probe may be differentially labeled, as described, for example, at page 44, lines 19-20, teaching three probes, each carrying a different fluorescent label. The Examiner's stated concerns about the possible formation of "probe dimers," hybridization stringency, and the like relate to experimental details that are easily controlled by one of ordinary skill in the art, and such artisan can readily practice the invention by causing probes to bind the polynucleotide template using nothing more than routine protocols.

Claim 50 is further rejected under 35 U.S.C. §112, second paragraph, for reciting the term "analogous" which the Examiner alleges is indefinite for failure to specify either the degree of homology and/or complementarity or whether the term which occurs twice in line 7 has the same meaning at all three noted locations. Applicant disagrees.

According to MPEP 2173.02 (Clarity and Precision) the definiteness of claim language must be analyzed, not in a vacuum, but in light of: (1) the content of the particular application

disclosure; (b) the teachings of the prior art; and (c) the claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made. The claim is to be considered as a whole to determine whether the claim apprises one of ordinary skill in the art of its scope and, therefore, serves the notice function required by 35 U.S.C. §112, second paragraph.

Claim 50 recites a target polynucleotide and an "analogous" wild-type polynucleotide Applicant submits that the word "analogous" as used in claim 50 is meets the threshold requirements of clarity and precision. The claim is directed to detecting a genetic polymorphism, which is the existence in the same population of two or more genotypes. The use of the term "analogous" to describe polynucleotides that differ as a result of the difference in nucleotide sequence that gives rise to the genetic polymorphism would be readily understood by one of ordinary skill in the art.

Claim 50 also recites a region on the target polynucleotide that comprises the genetic polymorphism; and a region on an analogous wild-type polynucleotide that is "analogous" to the region comprising the genetic polymorphism. Again, Applicant submits that the term "analogous" defines the subject matter with a reasonable degree of particularity and distinctness as one of ordinary skill in the art would understand which regions on the two analogous polypeptides. A skilled artisan reading the specification and the figures will readily understand that the mutant and wild-type polymorphism oligonucleotide probes recited in claim 50 bind at the respective regions that contain the polymorphism location, i.e., the "analogous" regions. E.g., the specification at page 17, lines 23-26, states that "[t]ypically the method for detecting genetic polymorphisms utilizes one "universal" oligonucleotide and two or more "polymorphism" oligonucleotides, one of which is a wild-type oligonucleotide used as a control." Thus, Applicant submits that the meaning of the term "analogous" as used in claim 50 is clear to one of ordinary skill in the art. MPEP 2173.02 states that "some latitude in the manner of expression and the aptness of terms should be permitted even though the claim language is nt as precise as the examiner might desire."

Claims 44 and 50 are rejected under 35 U.S.C. §112, as being indefinite in the recitation of "close proximity to one another." Applicant disagrees, and points out that the term "close proximity" is to be read in connection with the term "substantially adjacent." Claims 44 and 50 recite a university probe that is "substantially adjacent to an end of the polymorphism oligonucleotide probe so as to position the 5' leaving group and the 3' functional group in close proximity to one another." The specification at page 16, lines 2-15, is relevant:

> Oligonucleotides bind to a template or target polynucleotide "substantially adjacent" to each other when the 5' end of the "upstream" nucleotide binds to a base on the template or target polynucleotide that is directly adjacent to (see Figure 1), or within 2 bases either side of, preferably within about 1 base either side of, a base on the template or target polynucleotide bound by the 3' end of the "downstream" nucleotide. The term "substantially adjacent" thus includes, for example, oligonucleotides that are bound to the template or target polynucleotide directly adjacent to each other; oligonucleotides bound to the template or target polynucleotide such that there is a gap of 1 or 2 bases between the "upstream" and "downstream" oligonucleotide; and oligonucleotides bound to the template or target polynucleotide such that there is a 1 or 2 nucleotide overlap between the two oligonucleotides. When bound to a polynucleotide template or target substantially adjacent to each other, the upstream and downstream oligonucleotides self-ligate due to their *close proximity* and the presence of reactive groups on their adjacent ends (emphasis added).

The definiteness of claim language must be analyzed, not in a vacuum, but in light of the content of the particular application disclosure. MPEP 2173.02. Applicant submits that the phrase "close proximity to one another" is clear and precise.

Claims 44 and 50 are rejected under 35 U.S.C. §112, as being incomplete because there is no clear description at the end of the claim of how the claimed test result is realized. The Examiner also references claims 56, 64-74 and 78-80. Although Applicant disagrees the claims are incomplete, claims 44, 56, 64-66 and 70-72 (reciting a universal probe and a mutant

polymorphism probe) have been amended to recite "wherein the presence of an autoligated product indicates the presence of a genetic polymorphism in the target polynucleotide" (or "target RNA" in the case of claims 56 and 70-72); and claims 50, 67-69, 73 and 78-80 (reciting a universal probe, a wild-type polymorphism probe and a mutant polymorphism probe) have been amended to recite "wherein the presence of an autoligated product indicates the presence or absence of a genetic polymorphism in the target polynucleotide." In the method that utilizes both wild-type and mutant polymorphism oligonucleotide probes, the presence of an autoligation product comprising a mutant polymorphism probe indicates the presence of a genetic polymorphism, whereas the presence of an autoligation product comprising the wild-type polymorphism probe indicates the absence of a genetic polymorphism.

The Examiner also states alleges that with three probes present (e.g., claim 50), there must be at least two different probes labeled differently in order to determine the identity of all the possible autoligation products. This is not true. Although three different labels can be used (as exemplified in the specification and discussed above), detection of the different autoligation products can alternatively be accomplished without using detectable labels. E.g., at page 19, lines 12-15, the specification states that "[p]referably, at least one of the oligonucleotide probes is detectably labeled so that at least one of the ligation products is thereby labeled, although ligation events also can be detected using PCR, rolling circle amplification, gel electrophoresis or the like *without detectably labeling the ligation products*" (emphasis added).

The Examiner references the preamble to claims 44, 45, 47, 48, 50, 51, 53, 54, 56, 57, 59, 60, 64-74 and 76-80, stating that term "polymorphism" as recited therein suggests a test to find mutations, whereas the claims recite "mutant polymorphism" and "wild-type polymorphism" suggesting two or more different and conflicting meanings for "polymorphism." Applicant disagrees. As detailed above in response to the Examiner's rejection of claim 50 as being indefinite in its recitation of the term "analogous," the term "genetic polymorphism" is used in the art to describe the existence in the same population of two or more genotypes. Applicant has

chosen to term the more prevalent variant the "wild-type polymorphism" and the less prevalent variant the "mutant polymorphism." These are reasonable adjectives to choose to distinguish the two variants, and one of ordinary skill in the art would readily understand the intended distinction. As noted above, MPEP 2173.02 states that "some latitude in the manner of expression and the aptness of terms should be permitted even though the claim language is nt as precise as the examiner might desire."

The Examiner maintained the rejection of claims 44-48, 50-54, 56-60 and 64-80 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. The Examiner continues to object to the use of the term "comprising" in the claim as rendering the metes and bounds of the claim unclear. The rejection is respectfully traversed.

Applicant's detailed arguments in prior responses are reasserted but will not be reproduced here, and the Examiner is respectfully requested to reconsider the rejection in view thereof as well as the additional arguments presented below.

The present claims recite an "*oligonucleotide* comprising," at its 5' or 3' end, a "*nucleoside* comprising" a defined structural feature that allows it to participate in the autoligation reaction. Also recited in the claims is a resultant "autoligated oligonucleotide product comprising" two of the probes. Applicant asserts that the use of the term "comprising" in each of these instances clearly defines the metes and bounds of the claims.

Applicant submits that the Examiner's "routine" rejection of claims when the term "comprising" is directed to the structures of chemical compounds is misplaced when applied to the present claims. The Examiner uses the example "a compound comprising ethanol" to illustrate what the Examiner views as an inappropriate use of the transition term "comprising." However, the present claims do not recite "a *compound* comprising an oligonucleotide" or "a *compound* comprising a nucleoside." The basic structure of the probe is clearly that of an oligonucleotide, not some undefined "compound." Additionally, an oligonucleotide is

qualitatively different from a small molecule compound like ethanol. Ethanol has a unique molecular structure; the structure of an oligonucleotide however is one of lower resolution, i.e., a structure characterized by a polymerization of nucleotides. It is accordingly submitted that the recitation in a claim, for example, of an upstream oligonucleotide "*comprising*, as its 5' end, a nucleoside *comprising* a 5' leaving group" and a downstream oligonucleotide "*comprising*, as its 3' end, a nucleoside *comprising* a 3' functional group selected from the group consisting of a 3' phosphorothioate, a 3' phosphoroselenoate and a 3' phosphorotelluroate" (e.g., claim 44) clearly indicates to one of skill in the art the metes and bounds of the claims.

Moreover, Applicant submits that the open-ended term "comprising" is routinely accepted in claims issued by the USPTO, showing up in 3207 patents issued form 1976 through July 15, 2005, that recite in their claims "oligonucleotide comprising," "polynucleotide comprising," or "nucleic acid comprising." The Examiner himself is listed as primary examiner on 5 of these patents, four reciting "oligonucleotide comprising" and one reciting "nucleic acid comprising" as shown in the APPENDIX.

For at least the reasons described above, reconsideration and withdrawal of the rejection of claims 44-48, 50-54, 56-60 and 64-80 under 35 U.S.C. §112, second paragraph, is respectfully requested.

## Rejection 35 U.S.C. §102

The Examiner rejected claims 56-60 and 70-72 under 35 U.S.C. §102 as being anticipated by Letsinger et al. (U.S. Patent No. 5,780,613) (which the Examiner erroneously cites as "Lehninger" due to an apparent oversight). This rejection is respectfully traversed.

The Examiner states that this is a new rejection, and thus indicates no response to the Applicant's previous arguments are necessary. However, claims 56-60 and 70-72 were rejected under 35 U.S.C. §102 as being anticipated by Letsinger et al. (U.S. Patent No. 5,780,613), in the Office Action mailed April 22, 2004.

Claims 56-60 and 70-72 are directed to the detection of a genetic polymorphism in a *target RNA*. The claims recite "contacting the target RNA" with an oligonucleotide probe.

Applicant refers to the responsive arguments already of record at page 30 of Applicant's response mailed September 21, 2004. Applicant reiterates that Letsinger '613 does not teach a method for detecting a genetic polymorphism in a target RNA. Thus, the cited document fails to teach each and every element of the rejected claim.

Reconsideration and withdrawal of the rejection of claims 56-60 and 70-72 under 35 U.S.C. §102 as being anticipated by Letsinger et al. (U.S. Patent No. 5,780,613) is respectfully requested.

**Rejection under 35 U.S.C. §103**

The Examiner rejected claims 56-60 and 70-72 under 35 U.S.C. §103 as being unpatentable over Letsinger et al. (U.S. Patent No. 5,681,943) in view of Letsinger et al., (U.S. Patent No. 5,780,613) and further in view of Gryaznov et al. (U.S. Patent No. 5,571,903).

As noted above in the context of the rejection of these claims under 35 U.S.C. §102, the Examiner has failed to note that the claims 56-60 and 70-72 are directed to detection of a genetic polymorphism in a *target RNA*. It is respectfully submitted that the cited documents, either alone or in combination, do not teach or suggest, for example, the following elements of claim 56: a mutant polymorphism oligonucleotide probe that is complementary to a region on a target RNA that comprises the genetic polymorphism; a universal oligonucleotide probe capable of binding to a target RNA at a region that is conserved in the analogous wild-type RNA; contacting a target RNA with the universal oligonucleotide probe and the mutant polymorphism oligonucleotide probe to yield an autoligated oligonucleotide product comprising the universal oligonucleotide probe and the mutant polymorphism probe; and detecting the presence of the autoligated oligonucleotide product, wherein the presence of an autoligated product indicates the presence of a genetic polymorphism in the target RNA.

Reconsideration and withdrawal of the rejection of claims 56-60 and 70-72 under 35 U.S.C. §103 as being unpatentable over Letsinger et al. (U.S. Patent No. 5,681,943) in view of Letsinger et al. (U.S. Patent No. 5,780,613) and further in view of Gryaznov et al. (U.S. Patent No. 5,571,903) is respectfully requested.

## Withdrawal of Rejections

It is understood that all rejections that were set forth in the Office Action mailed April 22, 2004, and not repeated in the Office Action mailed February 14, 2005, are withdrawn.

## Claims Free of the Prior Art

It is understood that claims 44-48, 50-54, 64-69 and 73-80 are free of the prior art, as indicated at page 13 of the Office Action mailed February 14, 2005. The Examiner indicated that claims 44-48, 50-54, 64-69 and 73-80 would be allowable if rewritten or amended to overcome the rejection under 35 U.S.C. §112. Applicant respectfully submits that the rejection of claims 44-48, 50-54, 64-69 and 73-80 under 35 U.S.C. §112 has been overcome for reasons described above.

## Summary

It is respectfully submitted that the pending claims 44-48, 50-54, 56-60 and 64-80 are in condition for allowance and notification to that effect is respectfully requested. The Examiner is invited to contact Applicant's Representatives, at the below-listed telephone number, if it is believed that prosecution of this application may be assisted thereby.

Respectfully submitted for

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The undersigned hereby certifies that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR §1.10 on the date indicated above and is addressed to **Mail Stop Amendment**, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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